An insight into the feeding ecology of deep-sea canyon nematodes – Results from field observations and the first in-situ ^{13}C feeding experiment in the Nazaré Canyon

Jeroen Ingels[*,1], David S M Billett[2], Saskia Van Gaever[1,I], Ann Vanreusel[1]

[1]Marine Biology Department, Ghent University, Krijgslaan 281 S8, 9000 Ghent, Belgium, email: jeroen.ingels@ugent.be, ann.vanreusel@ugent.be

[2]National Oceanography Centre, Southampton, SO14 3ZH, United Kingdom, email: dsmb@noc.soton.ac.uk

[I]Present address: FOD Volksgezondheid, Veiligheid van de Voedselketen en Leefmilieu, Directoraat Generaal Leefmilieu, Sectie Marien Milieu, Place Victor Hortaplein 40 bus 10, 1060 Brussels, Belgium

*Corresponding author, email: jeroen.ingels@ugent.be, tel.: +32.(0)9.264.85.31, fax: +32.(0)9.264.85.98

Abstract

Submarine canyon systems provide a heterogeneous habitat for deep-sea benthos in terms of topography, hydrography, and the quality and quantity of organic matter present. Enhanced meiofauna densities as found in organically enriched canyon sediments suggests that nematodes, as the dominant metazoan meiobenthic taxon, may play an important role in the benthic food web of these sediments. Very little is known about the natural diets and trophic biology of deep-sea nematodes, but enrichment experiments can shed light on nematode feeding selectivity and trophic position. An in-situ pulse-chase experiment (Feedex) was performed in the Nazaré Canyon on the Portuguese margin in summer 2007 to study nematode feeding behaviour. ^{13}C-labelled diatoms and bacteria were added to sediment cores which were then sampled over a 14-day period. There was differential uptake by the nematode community of the food sources provided, indicating selective feeding processes. ^{13}C isotope results revealed that selective feeding was less pronounced at the surface, compared to the sediment subsurface. This was supported by a higher trophic diversity in surface sediments (Θ^{-1} = 3.50 ± 0.2) compared to the subsurface (2.78 ± 0.6), implying more food items may be used by the nematode community at the sediment surface. Predatory and scavenging nematodes contributed relatively more to biomass than other feeding types and can be seen as key contributors to the nematode food web at the canyon site. Non-selective deposit feeding nematodes were the dominant trophic group in terms of abundance and contributed substantially to total nematode biomass. The high levels of 'fresh' (bioavailable) organic matter input and moderate hydrodynamic disturbance of the canyon environment lead to a more complex trophic structure in canyon nematode communities than found on the open continental slope, and favours predator/scavengers and non-selective deposit feeders.

Keywords: pulse-chase experiment, stable isotopes, in situ, deep sea, meiofauna, biodiversity, trophic diversity

1. Introduction

Submarine canyons are one of the most pervasive features of ocean margins worldwide. They have been identified as important conduits for the transport of sediment and organic carbon (OC) from the shelf to bathyal and abyssal depths (Canals *et al.*, 2006; Palanques *et al.*, 2005), but they can also act as traps for carbon, making them important in calculating carbon budgets for shallow water and deep sea environments (Accornero *et al.*, 2003; Thomsen *et al.*, 2002). Near-shore primary production is transported to canyon heads by alongshore transport. Subsequently, tidal, gravity, and turbidity currents transport this material down canyon enriching the canyon system. Other inputs come from vertical transport from the euphotic zone and direct river discharges.

Meiobenthic densities and biomass generally show a strong positive correlation with the quality and quantity of organic matter (OM) reaching the deep-sea floor (Gooday, 2002; Gooday *et al.*, 1990; Lampadariou and Tselepides, 2006; Pfannkuche *et al.*, 1999). Nematodes are often considered important in the mineralisation of carbon in benthic ecosystems because of their numerical dominance within the metazoan meiobenthos, their relatively high reproductive capacities and metabolic rates, and their assumed intermediate trophic position. However, knowledge of nematode feeding ecology and their actual contribution to carbon mineralisation processes in the deep sea is poor (Soetaert *et al.*, 1997). In canyons, such as the Nazaré Canyon on the Portuguese continental margin, where sediment deposition rates and levels of bioavailable OM are high (Arzola *et al.*, 2008; de Stigter *et al.*, 2007; Garcia and Thomsen, 2008; Pusceddu *et al.*, 2010), meiobenthos might play an important role in benthic food webs. Ingels *et al*. (2009) concluded that nematode assemblages in the Nazaré Canyon showed particular adaptations (e.g. higher trophic complexity, variable nematode morphology, community shifts) in response to the greater supply of labile OM and the particular hydrodynamic conditions found in the canyon.

In deep-sea sediments, the numerical dominance of nematodes within the metazoan benthos is often translated into high standing stocks (biomass). Their high abundance and ubiquitous distribution render them very useful in experiments on small spatial scales which require sufficient replication. Nematodes, therefore, provide an ideal taxon for studying food-web structure and dynamics (Carman and Fry, 2002). Although the use of ^{13}C enriched carbon sources in experimental

pulse-chase setups has proven to be an effective way of unravelling the fate of organic C in deep-sea sediments (Buhring *et al.*, 2006; Levin *et al.*, 1999; Moodley *et al.*, 2002; Witte *et al.*, 2003a), deep-sea nematodes tend to be underrepresented in stable-isotope studies, mainly because large numbers of individuals need to be collected to obtain sufficient biomass for analysis.

The present study reports the results of the first in-situ ^{13}C pulse-chase experiment (Feedex) in a deep-sea canyon, focussing on nematodes. After in situ deposition of two types of labelled food (pelagic diatoms and benthic bacteria) we followed the nematode community's response at an organically enriched canyon site along the vertical sediment profile (0-1 and 1-2 cm) and through time in order to assess the ability and selectivity of the nematode community to use settled OM. Complementary field observations were used to understand any potential differences in uptake rates between food sources or sediment layers.

2. Material and Methods

2.1. Study site and sampling technique

The Nazaré Canyon is one of the largest submarine canyons in Europe (Fig. 1A). It cuts the shelf 500 m off the Portuguese coast and extends westwards for about 210 km (Tyler *et al.*, 2009; Vanney and Mougenot, 1981). Unlike many submarine canyons, the Nazaré Canyon is not connected to a large river system. However, because the canyon intersects a large part of the shelf it entraps large quantities of sediment moving along the Portuguese coast and so is recognised as a major sediment pathway among European canyons (Tyler *et al.*, 2009). Besides high rates of sedimentation, OM transport, entrapment, and burial, the Nazaré canyon is hydrodynamically very active and its heterogeneous topography contributes to a complex current regime within, and even beyond, the canyon's borders (Tyler *et al.*, 2009) with significant impacts on the canyon fauna (Curdia *et al.*, 2004; Garcia *et al.*, 2007; Ingels *et al.*, 2009; Koho *et al.*, 2007). Terraces in the middle canyon area are draped in thick layers of muddy turbidites creating a distinct depot centre with little sediment passing beyond the middle canyon section (de Stigter *et al.*, 2007). These terraces are ideal sites for sampling and experimentation; especially when studying the impact of highly elevated levels of sedimentary OM on the benthos.

Within the EC FP6 funded HERMES programme, several cruises were organised to investigate the Nazaré Canyon (Billett, 2006; Weaver, 2005; Weaver and Masson, 2007). Particular attention was given to a terraced site at ca. 3500 m depth (Fig. 1B). This site had been studied previously with regard to meiobenthos (Ingels *et al.*, 2009). For convenience we will use the same terminology as in Ingels *et al.*(2009) and refer to this site as Canyon Middle (CM). Station CM is situated on a

sediment-draped, terraced slope next to the V-shaped axial channel and is characterised by extremely high sediment accumulation rates (Arzola *et al.*, 2008) and an active hydrographic regime (de Stigter *et al.*, 2007). Highest sedimentary OM concentrations in the Nazaré Canyon were recorded at CM (Wolff *et al.*, submitted), resulting in dense and rich meiobenthic communities (Ingels *et al.*, 2009). The location was most suitable for in-situ experiments because 1) soft mud characterised the whole of the terrace, facilitating ROV use and the deployment of the experimental units (Fig. 1C, D), 2) there were high meiofaunal abundances, and 3) the hydrography of the area and biogeochemical properties of the sediment were well known. All experiment and background samples were taken by means of push cores (internal diameter of 57 mm, 25.5 cm² cross-surface area) manipulated by the ROV *ISIS*, during the RRS *James Cook* cruise 10 (13.05. – 07.07.2007; Weaver and Masson, 2007). The total seabed surface covered by the experimental setup and the background sampling was roughly 20 x 20 m.

2.2. Background samples

2.2.1. Meiofauna and nematode community structure

To characterise the meiofaunal community, three replicate ROV push cores were sliced (0-1, 1-2, 2-3, 3-4, 4-5 cm) and preserved in borax-buffered 4 % formalin. Sediment slices were subsequently rinsed over 1000- and 32-µm sieves to separate the meiofauna size class and centrifuged 3 times with the colloidal silica polymer Ludox HS (Heip *et al.*, 1985; Vincx, 1996). After staining with Bengal Rose, all metazoan meiobenthic organisms were classified at higher taxon level (following Higgins and Thiel, 1988) and counted under a stereomicroscope (50x magnification). About 100 to 150 nematodes were picked out randomly from each slice, transferred to glycerine (Seinhorst, 1959) and mounted on glass slides. Nematodes were identified to genus level under a compound microscope (1000x magnification) using the pictorial key to nematode genera of Platt & Warwick (1988), taxonomic data from the Ghent University library and the NeMys database (Deprez *et al.*, 2005, www.nemys.ugent.be). All identified individuals were classified into four feeding-type groups (selective deposit feeders (1A), non-selective deposit feeders (1B), epigrowth or epistrate feeders (2A), and predators/scavengers (2B)) following Wieser (1953). Nematode dimensions (length – excluding filiform tail tips, maximum width) were measured using a Leica DMR compound microscope and Leica LAS 3.3 imaging software. Nematode wet weight (wwt) biomass was calculated using Andrassy's formula (Andrassy, 1956), a dry-to-wet weight ratio of 0.25 was assumed, and Jensen's 12.4 % conversion factor (Jensen, 1984) served to translate nematode wwt into carbon weight. To calculate total biomass of each sediment layer, sampling station and feeding-type group, nematode biomass was averaged over each identified genus and multiplied by their

respective densities within the group defined (i.e. per sediment layer, sampling station and feeding-type group).

Based on Wieser's (1953) trophic groups, the nematode trophic diversity was calculated as the reciprocal of the trophic index (Θ) by Heip *et al.*(1998)), so that a higher Θ^{-1} value corresponds to a higher trophic diversity or complexity. For nematode structural diversity, H_0 (= genus richness) and H_2 (Hill, 1973) were calculated using genus relative abundance data.

2.2.2. Environmental variables

Three replicate ROV push cores were taken for granulometric, geochemical and natural sedimentary ^{13}C isotope analyses. They were analysed in 1 cm slices down to 5 cm sediment depth. Grain-size distribution was measured using a Coulter Counter LS 100^{TM} Particle Size Analyser and classified after Wentworth (1922). Following freeze-drying and homogenisation, the sample slices were acidified with dilute HCl until complete decarbonisation (liquid 1 % HCl was repeatedly added to the samples on a heated (80 °C) metal multiwell plate at 1-hour intervals (5 hours) until all carbonates were eliminated. Samples were then left overnight to dry on the heated plate in a fume cupboard to eliminate excess HCl). After acidification and drying, total sedimentary OC (TOC) and N content were measured using a Carlo Erba elemental analyser. For analysing the chloroplastic pigments, sediment samples were lyophilised and homogenised prior to extracting the pigments in 90 % acetone. Pigments were then separated using reverse phase HPLC, and measured with a Gilson fluorescence detector according to Wright & Jeffrey (1997). To assess the fresh OM of photosynthetic origin we applied the ratio between chlorophyll a (chl-a) content and the phaeopigments (Garcia and Thomsen, 2008; chl-a breakdown products, hereafter referred to as phaeo).

2.3. Experimental procedures

2.3.1. Cultivation and ^{13}C enrichment of food sources

The cosmopolitan pelagic diatom, *Skeletonema costatum* (average cell radius ca. 3 µm) was cultured axenically in f2 medium (Guillard, 1975) in sterile Erlenmeyer flasks at 16 – 18 °C with a 12:12-h light-dark period. Diatoms were enriched through addition of 5 ml $NaH^{13}CO_3$ (^{13}C, 99 %, Cambridge Isotope Laboratories; 336 mg per 100 ml milliQ H_2O). After labelling, the medium was rinsed away and the diatom cells were extracted by triple centrifugation (3500 rpm, 5 min), lyophilised and kept under dry atmospheric conditions prior to experimental use. This labelling technique resulted in an average $\delta^{13}C$ value of 107,087 ‰, equalling ca. 55 % ^{13}C.

Benthic bacteria were isolated from coastal sediment samples and were cultivated using a broth based on sterilised artificial seawater (ASW, salinity of 35 (Moens and Vincx, 1998)), 0.2 g l^{-1} Bacto Beef extract. Initially, regular D-glucose and ^{13}C-glucose were added at 0.7 and 0.3 g l^{-1}, respectively. Twenty-four hours before harvesting, an identical dose of ^{13}C-glucose was added to the culture to compensate for respiration loss of ^{13}C, ensuring efficient label uptake. During growth, the cultures were placed on a shaker (120 rpm) in an incubation chamber at 25 °C to stimulate growth. The bacteria were then harvested and rinsed through triple centrifugation in sterile ASW, lyophilised and kept under dry atmospheric conditions until use. Average δ^{13}C of the labelled bacteria was 27,895 ‰, implying nearly a quarter of their carbon was ^{13}C.

2.3.2. In-situ pulse-chase experiment

Nine Feedex units were deployed using a bottom lander system (Hughes *et al.*, submitted; Weaver and Masson, 2007). Once the lander was on the seabed, the units were collected by the ROV *ISIS* and pushed ca. 10 cm into the sediment (Fig. 1C, D). The units were located randomly on the bottom sediments, but positioned 4 to 6 m apart to ensure sufficient ROV working area around them without interfering with other units already deployed. Each unit consisted of a double-boarded wooden triangular frame fitted with 3 microcosm-sized tubes (diameter 10 cm) and was equipped with an injection module of 3 syringes (one for each tube). The syringes were operated simultaneously by a single action of the ROV's manipulators. In each unit, one syringe was filled with labelled bacteria, one with labelled diatoms (*Skeletonema costatum*), and one was left blank as control (natural nematode ^{13}C isotopes). For the labelled treatments, we projected an organic flux of 10 g C yr^{-1} m^{-2}, mimicking observational data (de Stigter *et al.*, 2007) and recalculated for the duration of the experiment. The projected C-flux translated into 3 mg C exp^{-1} $core^{-1}$, equivalent to ca. 15.1 and 24.1 mg of bacteria and diatom dry weight, respectively. The tubes were sealed at the top with a semi-permeable, finely perforated membrane (easily penetrable with ROV push cores) to avoid organic influx and biological disturbance during the experiment, yet still allowing gas exchange and thus avoiding oxygen depletion at the sediment water interface within the tube microcosms. Once the experiment was set up on the seabed labelled material was injected for all nine units. Sampling occurred at three time intervals: 1 (T1), 7 (T2) and 14 (T3) days after the incubations were started. At each of these time periods all the tubes in 3 randomly chosen units were sampled with push cores by the ROV; three units at T1, three at T2 and three at T3. It should be noted here that because of de-compaction of the sediment within the tube microcosms (caused by slight moving of experimental units in the sediment bed by manipulation of the ROV), not all replicate subsamples could be recovered. Loose sediment was sometimes seen falling out of the push cores upon retrieval

by the ROV's manipulator arm. The undisturbed push cores were sliced in 1 cm slices down to 2 cm sediment depth upon retrieval and stored at -20 °C until further analysis. In the present study we referred to the 0 – 1 and 1 – 2 cm sediment layers as 'surface' and 'subsurface', respectively.

2.4. Isotope analysis, data treatment and analysis

For natural ^{13}C isotope analysis of the sediment, only the top 2 cm was used. Aliquots (20 – 40 mg) of dried, ground and homogenised sediment were weighed and then acidified in pre-glown (overnight at 550 °C) Ag cups with dilute HCl (for method see section 2.2.2.) to eliminate the carbonate fraction. The cups were subsequently pinch closed and stored in Multi-well Microtitre plates under dry atmospheric conditions until analysis.

The frozen experimental samples (natural and enriched nematode samples) were thawed, rinsed over 1000- and 32-µm sieves, and centrifuged 3 times with Ludox to extract the meiofauna. The colloidal silica gel Ludox did not affect the ^{13}C signal of the nematodes as observed during laboratory tests (Moens, pers. comm.). No colouring or preservatives were used to avoid C contamination of the samples. After centrifugation, the separated meiofauna was rinsed with MQ water and processed immediately. For each sample 100 nematodes (when available) were handpicked with a fine sterile needle, rinsed twice in MQ water to remove adhering particles, and transferred to a drop of MQ water in 2.5 x 6 mm pre-glown (overnight at 550 °C to remove any contaminating OC) Al cups. These were then oven-dried overnight at 60 °C, pinched closed and subsequently stored under dry atmospheric conditions until analysis. We note here that the uptake values represent the nematode community as a whole (average of ca. 100 individuals), and are therefore a representation of different genera, feeding guilds and nematode life stages, all possibly displaying different feeding behaviour. Considering the high biomass requirements for a valid isotope analysis (i.e. a large number of small deep-sea nematodes are required to obtain sufficient biomass), distinguishing between feeding types and/or genera was not feasible because of the low availability of specimens per group and the relatively high generic diversity in the samples. The community level data allowed us to infer feeding behaviour for the nematode community on a whole, rather than focussing on smaller groups of nematodes that do not present the whole community.

The ^{13}C isotopic composition of sediment and nematode samples was determined with a PDZ Europa ANCA-GSL elemental analyser interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK; UC Davis). Minimal He dilution was applied for the low biomass nematode samples.

All nematode $\delta^{13}C$ isotope data were corrected with the average measurements of empty Al control cups. Uptake of ^{13}C is presented as excess ^{13}C (above natural abundance) and is expressed as total C uptake in the core (*I*) and as specific uptake ($\Delta\delta^{13}C = \delta^{13}C_{sample} - \delta^{13}C_{control}$). $\delta^{13}C_{sample}$ is calculated as $[(R_{sample} – R_{VPDB})/R_{VPDB}] \times 10^3$ with $R_{VPDB} = 0.0112372$ = the carbon isotope ratio of the Vienna Pee Dee Belemnite (VPDB) standard, and $R_{sample} = [(\delta^{13}C_{sample}/1000) + 1] \times R_{VPDB}$ (Craig, 1957). The fractional abundance of ^{13}C (*F*) equals R/(R + 1) and is used to calculate the excess ^{13}C (*E*) in the samples, which is the difference between the ^{13}C fraction of the sample (F_{sample}) and the ^{13}C fraction of the control ($F_{control}$): $E = F_{sample} - F_{control}$. Data are reported as the average of replicates with standard deviation. Although specific uptake ($\Delta\delta^{13}C$) gives a measure of excess ^{13}C, it does not account for the number of nematodes in the analysed sample, nor does it consider the quantity of C added at the start of the experiment. However, both are needed to make a sound comparison between the two treatments. For this purpose *I* (total C uptake, µg C $core^{-1}$) was calculated in the present study as the product of *E* and C-weight (µg) and was recalculated to total core C uptake by the nematode community present (µg C $core^{-1}$); C-weight of nematode samples was obtained through isotope analysis. We mention here that 3 replicates were not always available for the enrichment experiments, which impaired the use of statistical analyses. For the natural isotope values of the sediment and nematodes a t-test was applied to test for significant differences between surface and subsurface sediments.

3. Results

3.1. Environmental variables

Environmental variables and sediment properties are summarised in Table 1. The sediment grain distribution indicated high silt content. Only a minor fraction was sand. Sedimentary TOC levels averaged 2.0 ± 0.05 % and total nitrogen content ranged 0.21 – 0.23 %. Throughout the vertical sediment profile C:N remained relatively constant (8.8 – 9.3 %). CPE and chl-a:phaeo concentrations showed similar trends. Both decreased towards the 3 cm horizon, but at 3 – 4 cm depth there was a peak in concentration and 'freshness'. At 4 – 5 cm depth, CPE and chl-a:phaeo values equalled those from the subsurface.

3.2. Meiofauna and nematode structure, function, and diversity

Sixteen metazoan meiofauna taxa were identified (Table 2), of which nematodes were always the dominant group (> 90 %), followed by crustacean nauplii, polychaetes (predominantly larvae), and copepods. Other taxa comprised < 1 % of total meiofaunal abundance. The abundances of all taxa typically decreased with increasing depth in the sediment, except for polychaetes, for which

numbers remained relatively constant. Total metazoan meiofauna abundances were relatively high with an average of 1417 ± 102 ind. 10 cm^{-2}.

A total of 59 nematode genera were identified of which *Retrotheristus*, *Halalaimus*, *Dichromadora*, and *Molgolaimus* dominated with abundances > 5 %. The genus richness (H_0) per sediment slice is presented in Table 3, together with H_2, trophic diversity (Θ^{-1}), and relative abundance and genus richness of each trophic group. Genus richness and H_2 were highest in the second and third centimetre of the sediment, and lowest diversity was observed at 4 – 5 cm. In contrast, trophic diversity was greatest at the surface, and decreased down the vertical sediment profile to a depth of 4 – 5 cm, where it increased slightly. Non-selective deposit feeders displayed highest genus richness (20), followed by selective deposit feeders (18), epistrate feeders (14), and scavengers/predators (7).

Total core dry weight nematode biomass averaged 161 ± 65 µg 10 cm^{-2} (ca. 80 µg C 10 cm^{-2}). Biomass values for each feeding type and per sediment slice are shown in Fig. 2 (no replicates, pooled data). Generally, total nematode biomass decreased with increasing sediment depth, but there is a decrease in the abundance and average individual biomass (i.e. size) of predators/scavengers in the 1 – 2 cm sample, which affects total nematodes biomass in this layer. Below 2 cm sediment depth predator/scavenger abundance decreased and yet its biomass reached higher values than at the surface, indicating their mean size increases with increasing sediment depth. Furthermore, biomass of all other trophic groups decreased with sediment depth. Hence, predatory/scavenging nematodes contribute more than other feeding types to total biomass deeper in the sediment.

3.3. Natural ^{13}C isotopes and enrichment experiment

Fig. 3 shows the natural $\delta^{13}C$ signals of nematodes ($\delta^{13}C_{nema}$), bulk sedimentary OM ($\delta^{13}C_{sed}$), and the isotopic offset between nematodes and the sediment ($\Delta\delta^{13}C_{nema\text{-}sed}$) for the upper 2 centimetres of the sediment. Natural $\delta^{13}C_{nema}$ averaged -21.36 ± 2.0 ‰ and -17.89 ± 0.4 ‰ for 0 – 1 and 1 – 2 cm sediment depths, respectively (t-test, not significant, $p>0.05$). The $\delta^{13}C_{sed}$ values indicated that the surface sediment (0 – 1 cm, -22.20 ± 0.4 ‰) was significantly more depleted in ^{13}C compared to the subsurface (1 – 2 cm, -20.48 ± 0.9 ‰) (t-test, $p=0.03$). In all replicates an isotopic fractionation between sediment and nematodes was apparent with $\Delta\delta^{13}C_{nema\text{-}sed}$ averaging 0.71 ± 2.00 and 2.59 ± 0.47 ‰ for 0 – 1 and 1 – 2 cm layers, respectively (t-test, not significant, $p>0.05$).

The nematode community assimilated ^{13}C-enriched bacteria and diatoms in both the 0 – 1 and 1 – 2 cm layers of the sediment (Table 4, Fig. 4). Lack of replication prevented statistical comparisons between different time/sediment depth combinations.

The total uptake of the labelled ^{13}C by the nematode community per core was calculated as *I* (total C uptake, µg C core^{-1}) and these values are presented in Fig. 4. At the surface (0 – 1 cm) bacterial *I* reached a maximum after 14 days (T3, 0.22 µg C core^{-1}). In contrast, the nematode community in the 1 – 2 cm layer of the bacterial treatment showed very little uptake even after 14 days (T3, 0.02 µg C core^{-1}). Diatom uptake was apparent in both sediment depths with maximum *I* uptake values after 14 days. At the surface, bacteria seemed the preferred food source for nematodes inferred from the higher maximum *I* values. Yet, diatom uptake seemed greater initially (T1-T2). For the subsurface (1 – 2 cm) sediment, bacterial uptake occurred only after 2 weeks of incubation. Diatom uptake in the subsurface on the other hand did not exhibit such a time lag since *I* increased after day 1.

4. Discussion

4.1. Nematode feeding selectivity

Accounts of the feeding habits of free-living marine nematodes in the literature are based mainly on buccal cavity and pharyngeal morphology (Alongi and Tietjen, 1980; Jensen, 1987; Moens and Vincx, 1997; Wieser, 1953) and pertain mostly to shallow waters. Nematodes can use a broad range of microbial food and non-living detrital material and may specialize on, or have preferences for, different types of food resources. In the deep sea, benthic ecosystems are generally considered to be sustained largely by microalgal detritus settling from surface waters and associated bacterioplankton (Billett *et al.*, 1983; Gooday, 2002). For deep-sea nematodes, very few observations of actual feeding behaviour on phytodetrital components or microbial organisms have been reported and a high level of uncertainty remains about the diet of these organisms. This lack of knowledge hampers a realistic assessment of the role and importance of deep-sea nematodes in benthic energy flows, not only at the specific and generic levels, but also at trophic group and community levels. Feeding experiments, such as pulse-chase experiments, have proven successful in addressing feeding ecology questions (Ingels *et al.*, 2010; Moodley *et al.*, 2002; Witte *et al.*, 2003a; Witte *et al.*, 2003b), but nematode community results for the deep-sea remain variable.

Experimental results show that the natural $\delta^{13}C$ isotope values of the nematode community in the surface sediment (-21.36 ‰) were lower than in the subsurface (-17.89 ‰) and displayed higher variability (SD = 2.0 ‰ and 0.4 ‰ respectively, Fig. 3). The greater variation in the 0 – 1 cm layer suggests that the nematode community at the surface may feed on a wider range of food sources compared to the community in the subsurface. This is supported by the higher offset between $\delta^{13}C$ values of sediment and nematodes in the 1 – 2 cm layer than in the 0 – 1 cm layer, indicating that specific food sources from within the bulk TOC pool are used more selectively in the subsurface than

in the surface layers. These observations contradict Rudnick's (1989) hypothesis for shallow-water nematodes, stating that nematodes at the sediment surface use freshly-deposited particulate OM selectively, whereas the nematodes from deeper strata rely on a more stable and older supply of refractory OM. The high supply rate of OM from various origins to sediments in the Nazaré mid-canyon (Garcia and Thomsen, 2008; Wolff *et al.*, submitted) is likely to supply the surface benthos with a large amount of different food particles of variable nutritional value. Hence, the food available to nematodes at the surface will be more varied, but this does not necessarily entail a selective feeding process for the nematode community as a whole (although selecting the most nutritious particles would be a preferable strategy, a broad range of food items supplied to the surface may enable trophic specialisation and allow for extended niche segregation between trophic groups in the community). This is confirmed by the high nematode trophic complexity (Θ^{-1} = 3.57) at the surface compared to deeper layers, implying that a larger range of resources are used by the nematode community in the top cm compared to deeper sediments.

The maximum total core C uptake values (*I*) suggest that bacteria were preferred over diatoms by the nematode community at the surface. However, only after 14 days did the $\delta^{13}C$ differ substantially from background values (the single $\delta^{13}C$ T2 value was clearly lower than the high $\delta^{13}C$ T3 values). Such a delayed uptake may signify an indirect uptake by feeding on microbiota growing on the dead labelled bacteria; i.e. nematodes may prefer to consume live heterotrophic bacteria which had first utilised the labelled carbon. Comparable lags in bacterial response have been reported by Witte *et al.* (2003b) for deep-sea in situ experiments in the NE Atlantic. Benthic bacteria are considered key players in the benthic food web because of their capability of exploiting organic inputs. They usually dominate benthic biomass in the deep sea and are thought to be the primary agents through which detrital OM is remineralised (Deming and Baross, 1993; Turley, 2000). For deep canyon systems, elevated prokaryote abundances and highly diverse bacterial communities have been reported in the literature (Amaro *et al.*, 2009; Polymenakou *et al.*, 2008) suggesting bacteria may play an important role in canyon sediments. The main consumers of bacteria within the nematode community are the microvores (selective deposit feeders: 1A). These nematodes are characterised by a minute buccal cavity, restricting them to small particulate food or dissolved organic matter. In the top cm of the sediment this group constitutes nearly a quarter of the total nematode abundance indicating they may make a significant contribution to total community uptake. Also epigrowth feeders (2A) have been found to feed on microbiota by scraping them off solid surfaces or mucus threads with their teeth. However, there are indications that bacteria do not comprise a significant part of their diet and hence their contribution to bacteria consumption may be limited. In contrast, bacteria do contribute to the diet of some non-selective deposit feeders. Considering 43 % of the

total nematode community comprised this feeding type, they may be responsible for a substantial share of total community uptake.

Notwithstanding the differences to background values, the maximum uptake of bacterial label at the surface was still very low in terms of maximum total core C uptake (0.22 µg c core^{-1}). If we assume a similar carbon mineralisation rate as reported by Witte & Pfannkuche (2000) for the Arabian deep sea, then the maximum bacteria uptake by the nematode community constitutes < 0.01 % of total benthic remineralisation. This is likely to be a considerable underestimate because the values do not incorporate carbon that had been processed and lost through respiration (Moens *et al.*, 1999). However, even taking such underestimates in consideration, the nematode community contributed substantially less than 1 % to total remineralisation of the added bacterial carbon, a result similar to ex-situ experiments performed in Polar Regions (Ingels *et al.*, 2010; Moens *et al.*, 2007). In the 1 – 2 cm stratum, the nematodes only showed a small uptake after 2 weeks of incubation. Possibly, the experiment microcosm tubes excluded megafaunal and/or macrofaunal activity in the sediment, preventing the reworking of the bacterial label from the surface into the deeper sediment strata, consequently rendering it unavailable to the nematode community in deeper sediment layers.

Nematode uptake kinetics for the diatom treatment was different compared to the bacterial treatment. No delay in uptake was suggested by the data, nor were there clear differences between different incubation times. With a maximum *I* of 0.10 µg C core^{-1} after 2 weeks of incubation diatom uptake in the top cm of the sediment was less than half compared to the bacteria treatment, but the nematode community in the subsurface layer did assimilate the diatom label. A significant proportion of diatom production in the euphotic zone may sink to the seafloor (Tremblay *et al.*, 2006), constituting a substantial food source for the benthos. It is recognised that diatoms are an important food for non-selective deposit feeders (1B) and epigrowth feeders (2A), but they are not limited to these food sources (Moens and Vincx, 1997). The non-selective deposit feeders form the dominant trophic group (43 %) and together with the epistrate feeders they make up nearly two thirds of the total nematode community. It seems plausible that because of their large numbers, these two feeding type groups substantially contributed to the total observed diatom uptake.

The low *I* values indicate that only a small proportion of the labelled food sources was ingested or digested by the nematode community. The labelled food sources, however, do not directly mimic naturally occurring OM input in the Nazaré Canyon, which comprises a broad range of detrital food items. Consequently, providing just diatom-based detritus may be responsible for the attenuated response of the nematode community. In addition, the size of diatom cells may limit the number of

diatom-feeding nematodes that are large enough to consume the cells (Olafsson *et al.*, 1999). However, with a small average cell radius of ca. 3 µm, it seems unlikely that *Skeletonema costatum* was too big for these nematodes to consume. Nevertheless, some deep-sea nematodes with very small buccal cavities may have had difficulties taking up the diatom cells (selective deposit feeders), but these nematodes did not dominate the nematode community. In addition, the timing of the experiment during a period following the spring/summer phytoplankton bloom and the subsequent export of euphotic production meant that naturally occurring 'fresh' and bioavailable food items already present diluted the uptake of the labelled food. Moreover, high TOC and 'freshness' levels were present at the surface and in deeper sediment layers at station CM, resulting in high nematode abundance and standing stocks (Ingels *et al.*, 2009). Elevated OM content, in combination with high sedimentation rates and regularly occurring sediment resuspension, are likely to sustain high sedimentary food levels for longer periods of time. Consequently, the addition of a single type of food to a system already so abundant in food sources may result in an attenuated response of the nematode community.

4.2. Environmental conditions in the Nazaré Canyon and their influence on nematode trophic status

The Nazaré Canyon is characterised by complex and variable hydrodynamic conditions (de Stigter *et al.*, 2007; Tyler *et al.*, 2009), which affect meiofauna abundances and assemblage composition (Garcia *et al.*, 2007; Ingels *et al.*, 2009; Soetaert and Heip, 1995), but increased food supply may act to increase abundances (Ingels *et al.*, 2009). The abundance of meiobenthic communities in canyons reflects the prevailing current regime to an extent, with often very low densities in areas where sediments are disturbed regularly and hydrodynamic activity is greatest, e.g. in the axis of a canyon (Bianchelli *et al.*, 2008; Garcia *et al.*, 2007; Ingels *et al.*, 2009; Van Gaever *et al.*, 2009). Although strong near-bottom flow can reduce the abundance and alter the assemblage of both meiofauna and macrofauna in the deep sea (Thistle and Levin, 1998), meiofauna appear to recover more quickly than macrofauna with regards to physical disturbance (Lee *et al.*, 2001a; Lee *et al.*, 2001b; Thistle *et al.*, 1999). This is especially the case for nematodes, most likely because of their trophic versatility as a group and their often opportunistic resilience to harsh environmental conditions. However, to what extent hydrodynamic activity alters the trophic composition of nematodes is not well understood.

The non-selective deposit feeders (1B) formed the dominant group (abundance and biomass) at station CM, but did not contribute as great a proportion of the different feeding types as observed for slope sediments (Ingels *et al.*, 2009). In contrast, Garcia *et al.* (2007) found that non-selective deposit feeders were more important in canyons than on the continental slope. The localities

studied by Garcia *et al.* (2007) were much more disturbed (near canyon axis) compared to the sediment draped terrace studied here and so we suggest that intense recurrent hydrodynamic activity at their sites is (partially) responsible for this discrepancy. In addition, non-selective deposit feeders have been known to exhibit recolonisation ability after severe disturbance, but in less disturbed environments they are less dominant (Lee *et al.*, 2001a). This is illustrated by the example of the genus *Sabatieria*, a nematode genus which co-dominates continental margin sediments (Vanreusel *et al.*, 2010). Analogous to reports by Lee *et al.* (2001b), Soetaert & Heip (1995), and Van Gaever (2009), *Sabatieria* was highly dominant in highly disturbed areas in the Nazaré Canyon (Garcia *et al.*, 2007), but at the more quiescent CM site, *Sabatieria* was present only in much lower abundances.

Higher relative numbers of predators and scavengers in the canyon compared to the continental slope (Ingels *et al.*, 2009) contributes to higher trophic diversity in the canyon and may reflect the greater amounts of food available (Huston, 1994). In addition, predatory or scavenging nematodes are naturally larger compared to other feeding types, which makes them more agile in disturbed canyon sediments and enhances their survival rate. The high biomass values of the predator/scavenger group (Fig. 2) could reflect their dominance in energy transfer within the community, and signifies their importance in the trophic food web. Another possible contributing factor to the success of this trophic group may be the low abundance of macrofaunal predators at station CM (M. Cunha, unpublished results). In the absence of macrofaunal predation pressure, the resulting high nematode standing stock at CM could sustain the success of the predatory/omnivorous group within the nematode community.

Whilst the majority of OM is captured in the upper Nazaré Canyon, tidal currents and intermittent gravity flows transport this material through the canyon and to the abyssal plain (de Stigter *et al.*, 2007). The diminishing strength of tidal currents with increasing depth leads to focussed sediment deposition in the middle canyon, were site CM is located. Although the OM in the deeper sections of the canyon is less bioavailable compared to the head of the canyon, it is still 'fresher', and present at much greater concentrations, than on the open slope (Garcia and Thomsen, 2008). As a result of decomposition of these high levels of OM, the sediments at CM rapidly become anoxic (Wolff *et al.*, submitted). Evidence for this comes from the high concentrations of elemental sulphur in these sediments. Reduced anoxic conditions and disturbed environments have been associated with nematode assemblages typified by genera such as *Sabatieria* (Soetaert and Heip, 1995; 2009; Vanreusel, 1990), which is present in greater numbers at station CM than at the adjacent slope (Ingels *et al.*, 2009).

4.3. Trophic and structural diversity in the Nazaré Canyon

There are many reports in the literature on nematode diversity. These are, however, mainly restricted to structural diversity on various spatial and temporal scales, or in relation to contrasting environmental settings. Rarely do studies combine structural as well as trophic diversity or investigate the intricate relationship between both (e.g. Danovaro *et al.*, 2009). Although the use of Wieser's (1953) scheme for nematode feeding type classification is widely used, it suffers some shortcomings when compared with empirical evidence (Moens and Vincx, 1997). The use of isotope techniques provides a significant advance in nematode deep-sea feeding ecology studies. Yet, the apparent lack of relation between Wieser's feeding-type groups and the actual niche these groups occupy still causes confusion.

Structural diversity (H_2) did not show a clear trend down the vertical sediment profile, but total number of genera (H_0) and evenness (H_2) were higher in the subsurface compared to the surface. Structural diversity did not correspond with trophic diversity, which decreased down the vertical profile (Table 3). This indicates that the nematode community becomes more dominated by certain trophic groups in deeper sediment layers that feed selectively on certain food sources. This corresponds with the lack of difference between nematodes and sediment ^{13}C signatures in surface sediments, which suggests that nematodes do not feed selectively, as opposed to subsurface nematodes which do.

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Fig. 1. Overview of the Western Iberian Margin with the studied area in the Nazaré Canyon (ca. 3500 m water depth); adapted from Weaver et al (2005). B: Detailed bathymetrical map representing the local geomorphological setting of the experimental site; bathymetry data compiled from a variety of sources, courtesy NOCS and Instituto Hydrografico, Lisbon; 30 kHz TOBI sidescan sonar data, courtesy NOCS; both data sets published by Lastras et al. (2009); Δ: experimental site.

Fig. 2. Vertical profile of total nematode dry weight biomass and dry weight biomass per trophic group (Wieser, 1953). Biomass values are based on pooled data per slice (pooled replicates; n = 473, 677, 322, 102 for 1A, 1B, 2A, 2B, respectively). 1A (selective deposit feeders), 1B (non-selective deposit feeders), 2A (epistratum feeders), 2B (predators/scavengers).

Fig. 3. Natural ^{13}C isotope signatures ($\delta^{13}C$) for sedimentary organic carbon ($\delta^{13}C_{sed}$) and nematode community (ca. 100 individuals per sample, $\delta^{13}C_{nema}$). The isotopic shift between $\delta^{13}C_{nema}$ and $\delta^{13}C_{sed}$ is represented by $\Delta\delta^{13}C_{nem\text{-}sed}$.

Fig. 4. Average uptake of experimentally labelled C by the total core nematode community during the experiments for both treatments. *I* is here calculated as the product of *E* and C-weight (µg) and was recalculated to total core C uptake by the nematode community (µg C $core^{-1}$) during the experiment for both treatments. All bars denote average ± SD.

Table 1. Environmental variables per sediment slice at the sampling site.

Sediment depth (cm)	Clay (%)	Silt (%)	Sand (%)	TOC (%)	TN (%)	C:N	CPE(µg.g^{-1})	Chl -a:phaeo
0-1	14.0 ± 0.8	76.4 ± 0.9	9.6 ± 0.4	2.00 ± 0.23	0.22 ± 0.04	9.1 ± 1.3	1.61 ± 0.13	0.19 ± 0.01
1-2	15.0 ± 0.4	75.0 ± 1.0	10.0 ± 0.8	1.98 ± 0.05	0.21 ± 0.00	9.2 ± 0.4	1.45 ± 0.30	0.18 ± 0.03
2-3	15.9 ± 1.2	73.2 ± 2.6	10.9 ± 1.5	1.92 ± 0.04	0.22 ± 0.01	8.8 ± 0.1	1.32 ± 0.21	0.14 ± 0.02
3-4	15.4 ± 0.7	74.2 ± 0.7	10.5 ± 0.4	1.99 ± 0.12	0.21 ± 0.01	9.3 ± 0.6	2.01 ± 0.84	0.62 ± 0.49
4-5	15.3 ± 1.3	75.0 ± 2.5	9.7 ± 1.2	2.07 ± 0.00	0.23 ± 0.03	9.1 ± 0.9	1.45 ± 0.20	0.26 ± 0.13

Table 2. Relative abundances (%) of metazoan meiofauna taxa per sediment layer (Total: ind. 10 cm^{-2}, average of replicates ± standard deviation).

	0-1 cm	1-2 cm	2-3 cm	3-4 cm	4-5 cm
Aplacophora	-	0.03 ± 0.05	-	-	-
Bivalvia	0.07 ± 0.07	0.03 ± 0.05	-	-	-
Gastrotricha	0.15 ± 0.12	0.03 ± 0.05	-	-	-
Halacarida	-	-	0.03 ± 0.06	-	-
Harpacticoidea	4.71 ± 0.68	2.37 ± 2.05	0.32 ± 0.28	0.50 ± 0.63	0.13 ± 0.22
Holothuroidea	0.02 ± 0.04	0.0 ± 0.05	-	-	-
Isopoda	0.02 ± 0.04	-	-	-	-
Kinorhyncha	1.28 ± 0.49	0.90 ± 0.56	0.25 ± 0.30	-	-
Nauplii	9.60 ± 2.64	2.01 ± 1.45	1.61 ± 1.39	2.20 ± 1.87	0.26 ± 0.30
Nematoda	83.42 ± 3.16	93.36 ± 2.56	95.38 ± 1.58	95.92 ± 1.52	96.23 ± 0.21
Oligochaeta	0.02 ± 0.03	0.06 ± 0.10	-	-	-
Polychaeta	0.58 ± 0.20	1.11 ± 1.01	1.65 ± 1.17	1.39 ± 0.65	3.06 ± 0.68
Priapulida	0.03 ± 0.06	-	0.03 ± 0.06	-	-
Tanaidiacea	0.09 ± 0.10	0.08 ± 0.09	0.56 ± 0.81	-	-
Turbellaria	-	-	-	-	0.13 ± 0.22
Other Metazoa	-	-	0.16 ± 0.28	-	0.20 ± 0.34
Total	640 ± 133	327 ± 254	226 ± 191	113 ± 71	112 ± 82

Table 3. Diversity values (Genus richness (H_0), Hill's diversity index (H_2), trophic diversity (Θ^{-1})) for the nematode community per sediment layer (left) and relative abundances and genus richness per trophic group for each sediment layer (right). Non-bracketed diversity values denote mean of 3 replicates ± SD; bracketed values below are based on pooled data (i.e. replicates were joined to calculate diversity values). Relative abundances and genus richness per trophic group for each sediment layer are also based on pooled data. 1A (selective deposit feeders), 1B (non-selective deposit feeders), 2A (epistratum feeders), 2B (predators/scavengers).

Sediment depth	H_0	H_2	Θ^{-1}	Feeding groups (relative abundance – genus richness)			
				1A	1B	2A	2B
0-1 cm	26.0 ± 2.6 (36)	10.97 ± 2.9 (14.62)	3.50 ± 0.2 (3.57)	0.24 - 12	0.38 - 16	0.25 - 4	0.13 - 4
1-2 cm	26.3 ± 5.5 (43)	11.98 ± 0.5 (18.79)	2.78 ± 0.6 (3.00)	0.19 - 12	0.47 - 17	0.27 - 9	0.07 - 5
2-3 cm	24.0 ± 9.5 (42)	10.79 ± 2.9 (19.76)	2.84 ± 0.4 (2.85)	0.28 - 12	0.49 - 16	0.16 - 9	0.07 - 5
3-4 cm	20.3 ± 2.1 (34)	7.90 ± 3.1 (12.07)	2.59 ± 0.2 (2.80)	0.35 - 9	0.44 - 14	0.19 - 8	0.02 - 3
4-5 cm	17.7 ± 3.8 (27)	5.52 ± 4.4 (8.98)	2.20 ± 0.7 (2.99)	0.39 - 5	0.37 - 13	0.21 - 5	0.03 - 4
Total	44.33 ± 3.1 (59)	15.15 ± 1.9 (21.67)	3.16 ± 0.3 (3.12)	0.29 - 18	0.43 - 20	0.21 - 14	0.06 - 7

Table 4. $\delta^{13}C_{nema}$ (‰) values of control and treatment samples (based on max. 100 nematode individuals). Rep: replicate, bracketed values denote µg C in the analysed sample.

Nazare Canyon	Sediment depth (cm)	Time (days)	Rep 1	Rep 2	Rep 3
Control	0-1	1	-20.97 (5.3)	-22.74 (3.4)	-
		7	-19.19 (7.0)	-	-
		14	-23.03 (2.8)	-	-
	1-2	1	-18.18 (3.3)	-	-
		7	-17.60 (5.0)	-	-
		14	-	-	-
Diatom treatment	0-1	1	-23.95 (6.7)	-9.47 (7.0)	-
		7	-19.52 (7.6)	-	-
		14	-22.66 (8.6)	-	-
	1-2	1	22.52 (7.1)	18.77 (3.9)	-
		7	9.17 (5.5)	-	-
		14	76.96 (4.4)	-	-
Bacteria treatment	0-1	1	-23.83 (5.3)	-2.83 (3.0)	-13.00 (4.3)
		7	-14.58 (8.4)	-	-
		14	46.76 (8.2)	52.45 (3.3)	-
	1-2	1	-20.10 (6.7)	-	-
		7	-17.28 (7.6)	-	-
		14	-15.28 (8.7)	-5.40 (6.3)	-

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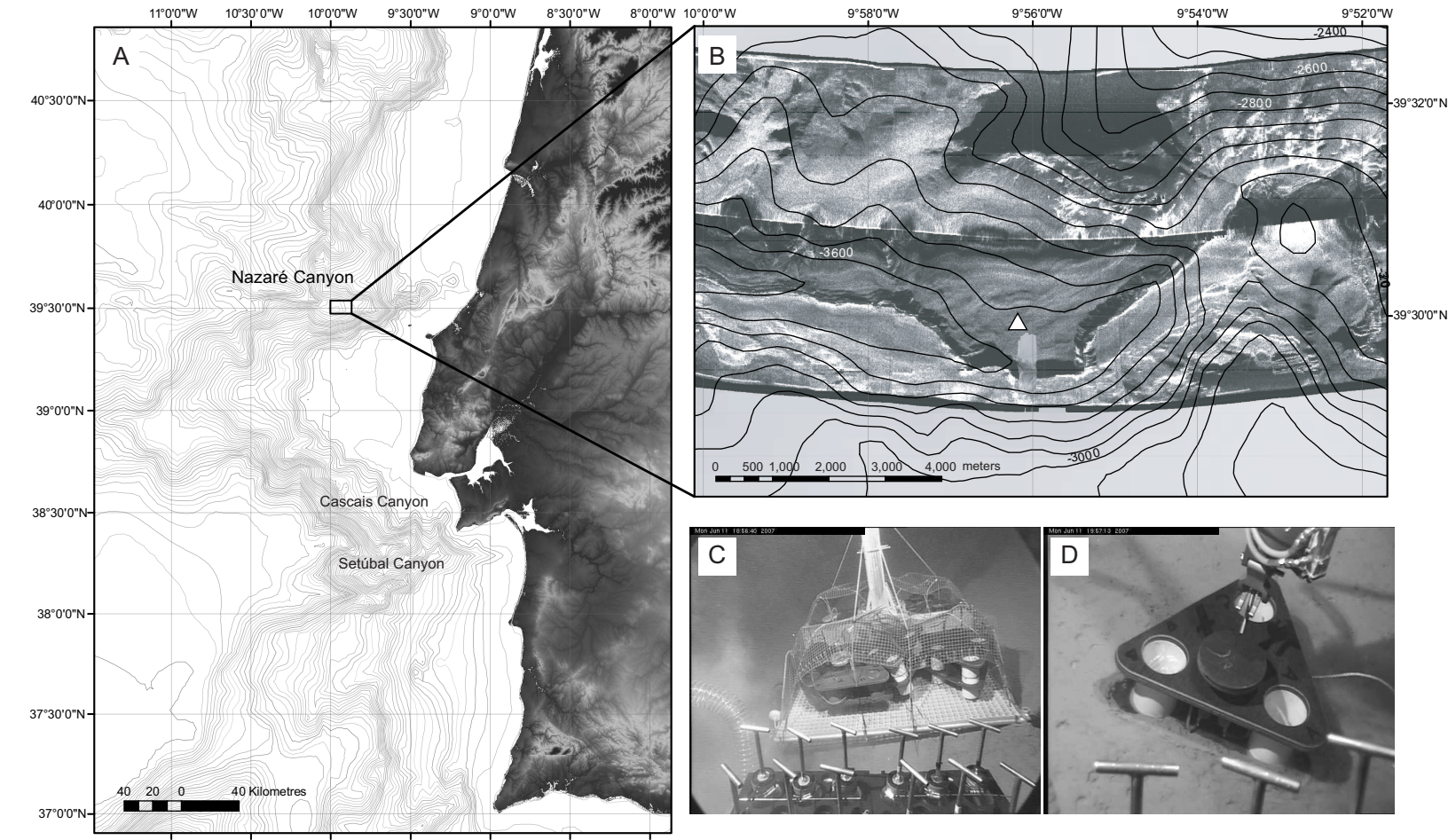
Figure 1


A
B
C
D
11°0'0"W
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8°0'0"W
40°30'0"N
40°0'0"N
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39°0'0"N
38°30'0"N
38°0'0"N
37°30'0"N
37°0'0"N
Nazaré Canyon
Cascais Canyon
Setúbal Canyon
40 20 0 40 Kilometres
10°0'0"W
9°58'0"W
9°56'0"W
9°54'0"W
9°52'0"W
39°32'0"N
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-3600
-3000
0 500 1,000 2,000 3,000 4,000 meters

Figure2

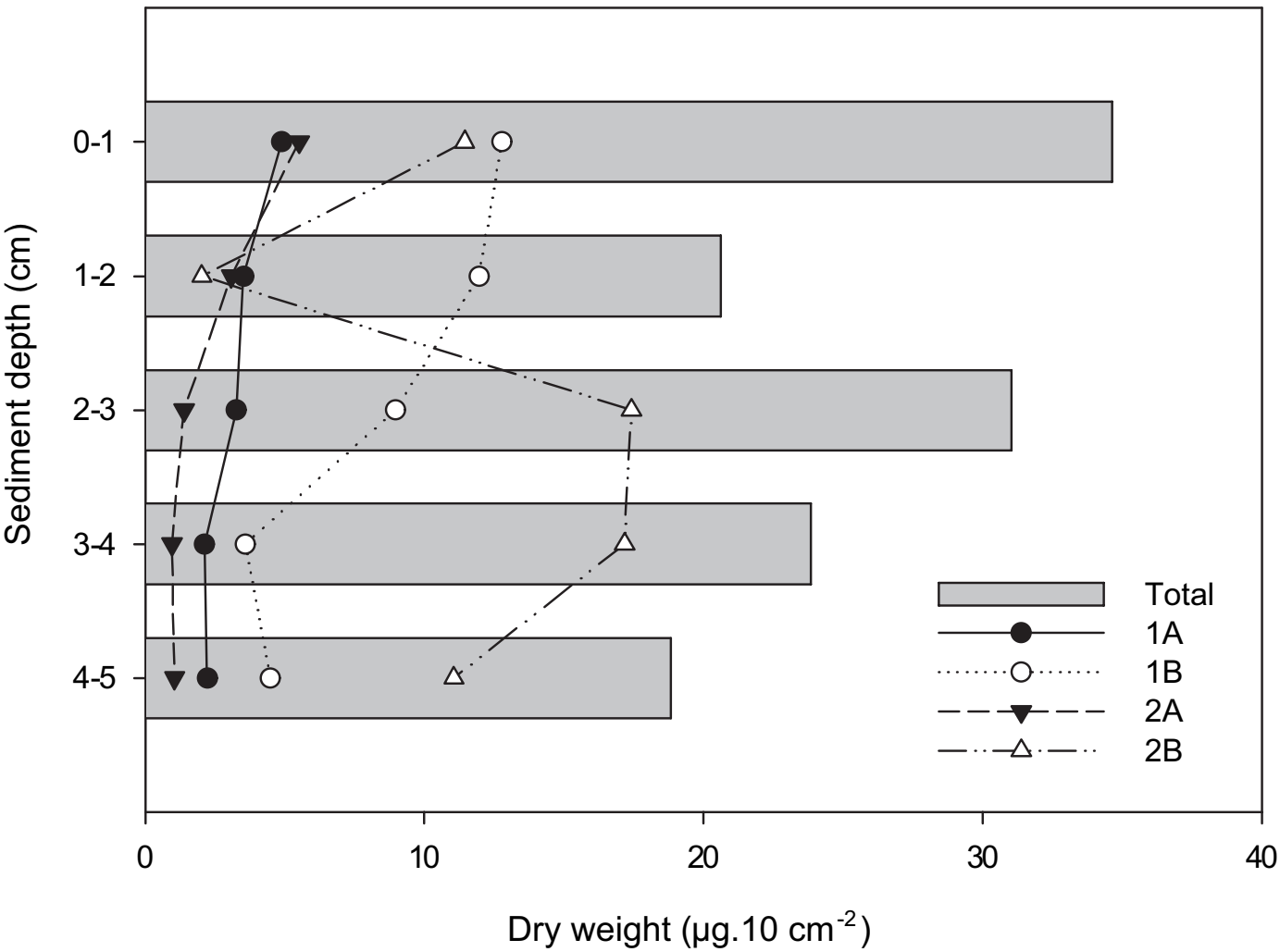

Sediment depth (cm)
0-1
1-2
2-3
3-4
4-5
0
10
20
30
40
Dry weight (µg.10 cm-2)
Total
1A
1B
2A
2B

Figure3

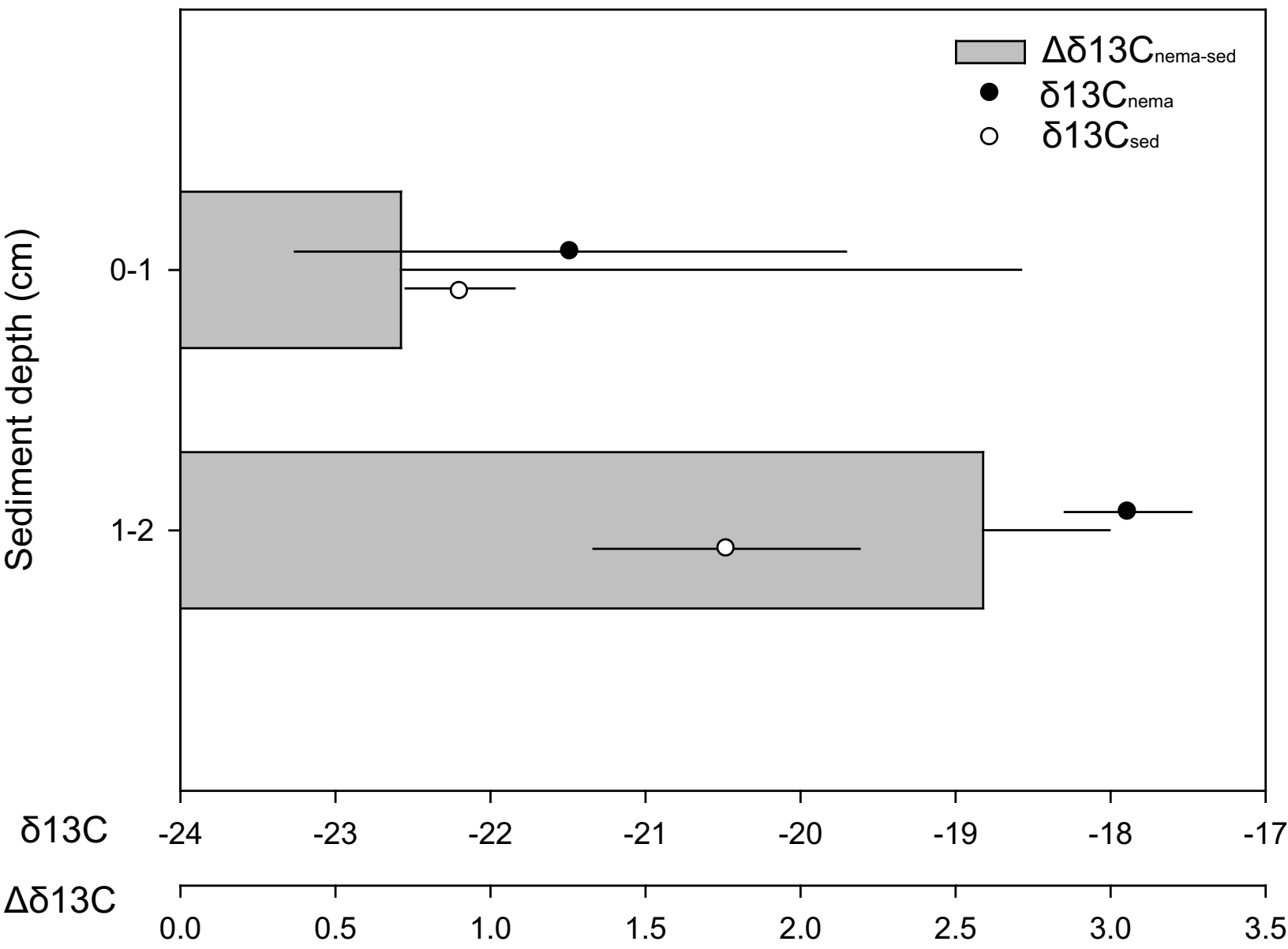
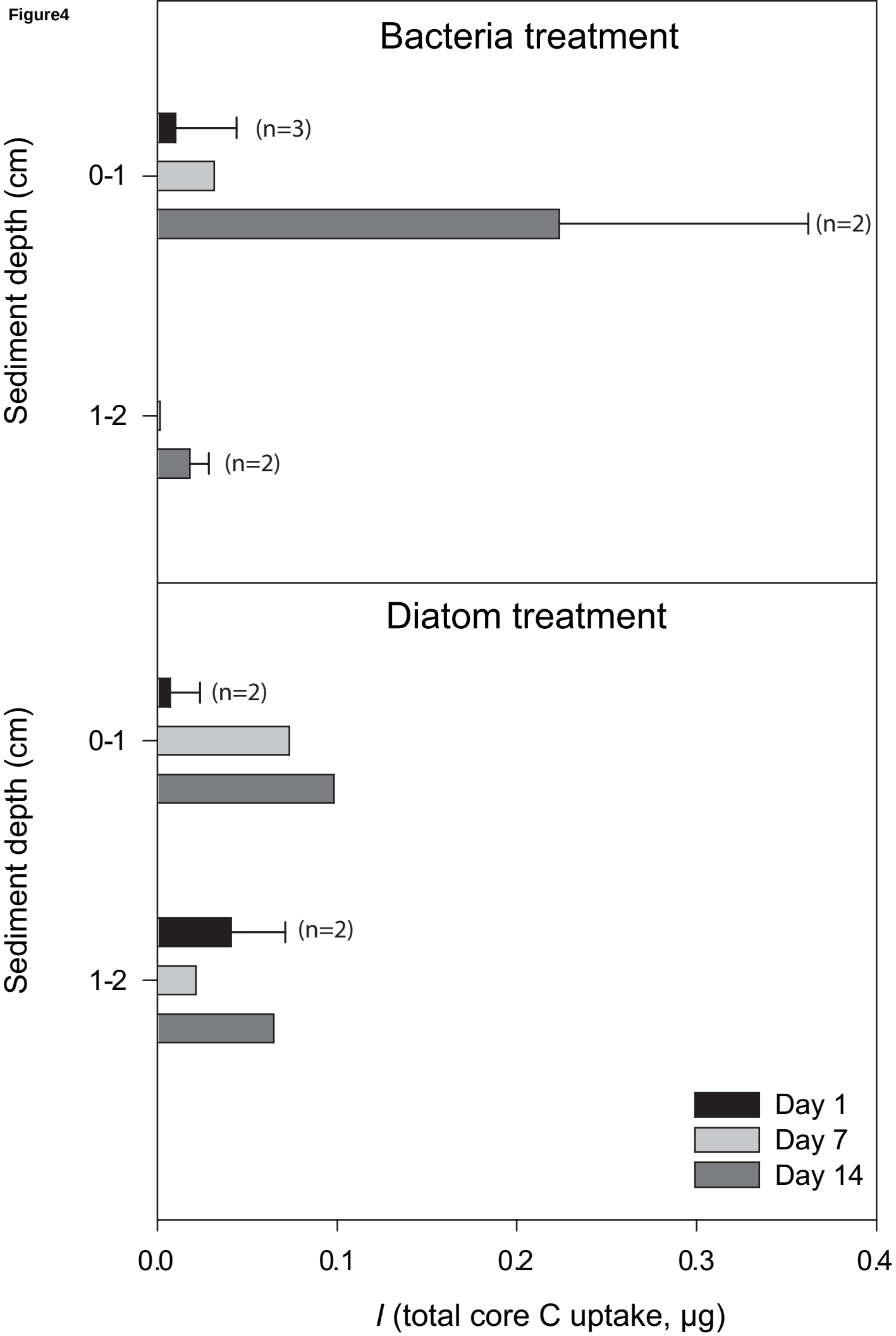

Δδ13Cnema-sed
δ13Cnema
δ13Csed
Sediment depth (cm)
0-1
1-2
δ13C
-24
-23
-22
-21
-20
-19
-18
-17
Δδ13C
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5

Figure4


Bacteria treatment
(n=3)
0-1
(n=2)
1-2
(n=2)
Sediment depth (cm)
Diatom treatment
(n=2)
0-1
(n=2)
1-2
Sediment depth (cm)
Day 1
Day 7
Day 14
0.0
0.1
0.2
0.3
0.4
I (total core C uptake, µg)